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ARTICLE

Transition Metals and Breast Cancer

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Increased levels of transition metals like iron, nickel, chromium, copper and lead are closely related to free radical generation, lipid peroxidation, formation of DNA strand breaks and tumor growth in cellular systems. In order to determine the correlation to malignant growth in humans, we investigated the accumulation of heavy metals in 8 healthy breast tissue samples and 20 breast cancer biopsies. The concentration of transition metals in breast tissue samples was assessed by a standardized AAS technique with acidic hydrolysis for sample preparation. Additionally, heavy metal analysis in all control biopsies has been performed by means of an ICP-MS technique. For statistical analysis of the results the Mann-Whitney U test was used. A highly significant accumulation of iron ($p < 0.0001$), nickel ($p < 0.00005$), chromium ($p < 0.00005$), zinc ($p < 0.00001$), cadmium ($p < 0.005$), mercury ($p < 0.005$) and lead ($p < 0.05$) was recorded in the cancer samples when compared to the control group. Copper and silver showed no significant differences to the control group whereas tin, gold and palladium were not detectable in any biopsies. These data suggest that non-physiological gradual accumulation of transition metals in the breast tissue may be closely related to the malignant growth process. (Pathology Oncology Research Vol 13, No 1, ???–???)

Key words: breast cancer, iron, nickel, chromium, mercury, cadmium

Introduction

Reports in the last two decades are closely relating the presence of transition metals like iron or copper to free radical generation via Fenton/Haber-Weiss reactions, ascorbate autoxidation, lipid peroxidation processes and formation of DNA strand breaks.[1-4] In turn, lipid peroxidation-induced malondialdehyde-DNA adducts can accumulate and reach high levels in the breast tissue of women with breast cancer, leading to endogenous DNA modifications.[5] Furthermore, ferric-EDDA- and -NTA complexes were proved to induce free radicals and renal carcinomas in Wistar rats, demonstrating the key role of transition metals in the abnormal proliferation process.[6,7] As repeated mitochondrial and nuclear DNA mutations may lead to malignant growth, we investigated the heavy metal content in breast cancer biopsies supplied by the Institute of Pathophysiology of Charles University in Prague.

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Abbreviations

EDDA: ethylenediamine N,N'-diacetate, NTA: nitrilotriacetic acid, AAS: atomic absorption spectrophotometry, ICP-MS: inductive coupled plasma – mass spectroscopy, MELISA: memory lymphocyte immunostimulation assay

Material and Methods

Heavy metal analyses have been performed in 20 frozen breast cancer biopsies and in 8 healthy breast tissue samples, supplied by the Dept. of Oncology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic and by Caritas Hospital St. Josef, Regensburg, Germany. The basic histopathologic characteristics of the investigated tumors are described in *Table 1.*

Table 1. **Basic histopathologic characteristics of breast tumors**

Histologic type	
Ductal carcinoma	12
Lobular carcinoma	4
Other	4
Grade	
I	5
II	12
III	1
Unknown	2
Hormone receptor status	
ER+	13
ER-	7
ER unknown	0
PR+	17
PR-	1
PR unknown	2
HER-2/neu staining intensity	
HercepTest 0	1
HercepTest 1	6
HercepTest 2	3
HercepTest 3	3
ND	7

ND: not done

The study was approved by the local ethic committee and all participants gave their informed written consent before enrollment in the study.

The concentrations of iron, cadmium, lead, chromium, tin, nickel, copper, mercury, silver, gold, palladium and zinc in the biopsy material have been measured by a standardized furnace-AAS technique using a Perkin Elmer Sima 6000 AA-spectrophotometer and acidic hydrolysis as pulping procedure for sample preparation.[8] Additionally, heavy metal analysis in all control biopsies has been done by using an ICP-MS technique in the Laboratory for Micro Trace Minerals, Hersbruck, Germany. All biopsies have been taken from the center of the tumor nodules, and metal concentration in 1 g of tumor breast tissue was measured and compared to metal concentration in the same amount (1 g) of healthy breast tissue. All tests have been performed three times and the final result per sample is expressed in mg/kg breast tissue, recording the mean value of three determinations. Mann-Whitney U test was used for statistic analysis of the results.

Results

Data analysis shows a highly significant accumulation of iron, nickel, chromium, cadmium, mercury, zinc and, to a lesser extent, of lead in malignant breast tissue, when compared to healthy breast tissue. Iron levels showed a dramatic increase in the breast cancer biopsies (median: 53173.5 µg/kg, range: 14664-205930 µg/kg) when compared with the control group (median: 10937 µg/kg, range: 5331-21646 µg/kg) ($p < 0.0001$) (*Fig. 1*). A strong nickel accumulation (median: 994.5 µg/kg, range: 469-3361 µg/kg) was recorded in the patient biopsies. Control biopsies showed measurable levels (median: 21 µg/kg, range: 11-33

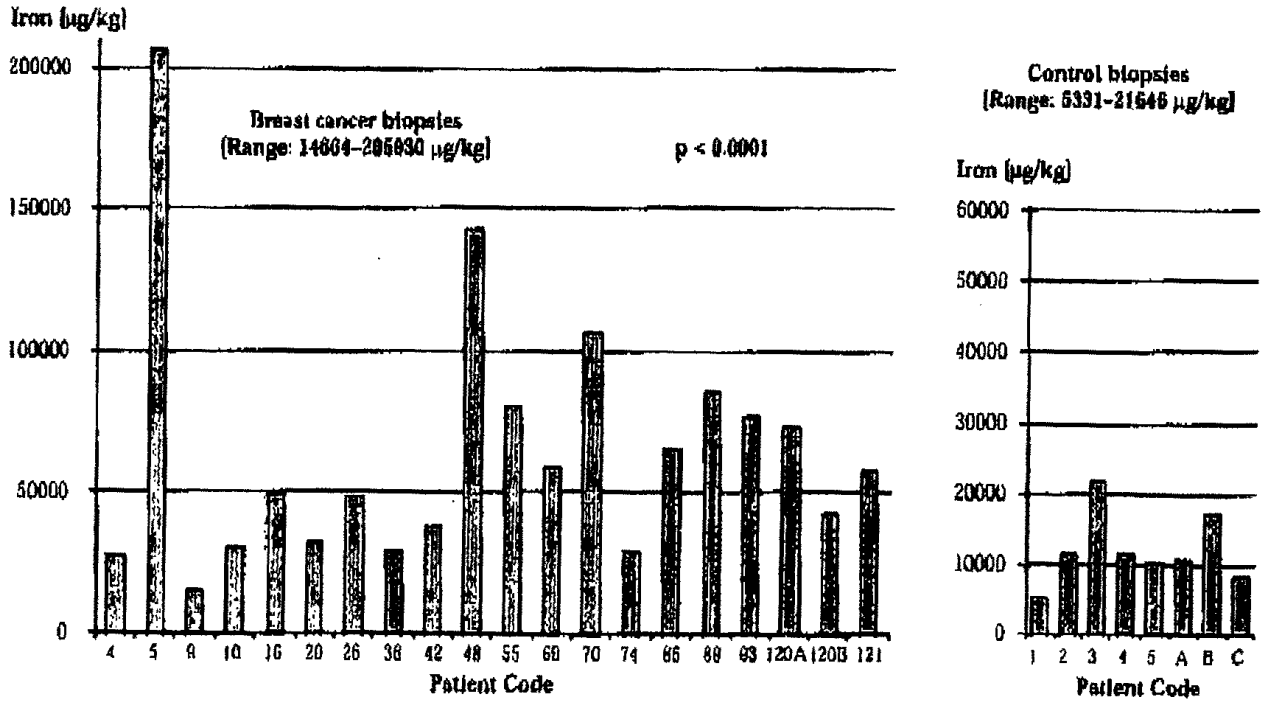

Figure 1. Iron content of 20 breast cancer and 8 control human biopsies. Furnace-AAS technique

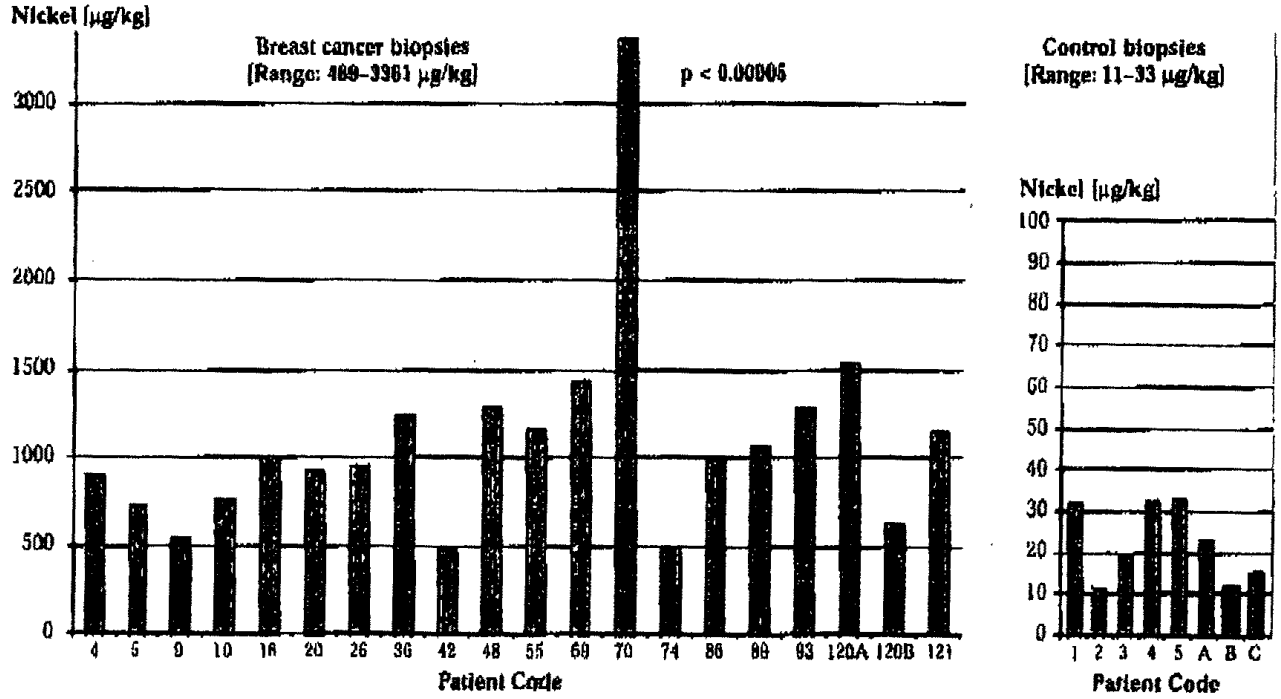

Figure 2. Nickel content of 20 breast cancer and 8 control human biopsies. Furnace-AAS technique

µg/kg), but at more than one order of magnitude lower ($p < 0.00005$) (*Fig. 2*). Similar results have been noticed for chromium (median: 815.5 µg/kg, range: 313-5978 µg/kg) when compared with the control group (median: 38.5 µg/kg, range: 19-119 µg/kg) ($p < 0.00005$) (*Fig. 3*).

A surprisingly high accumulation of zinc (median: 17075 µg/kg, range: 1326-97895 µg/kg) was recorded in the cancer biopsies, the difference from the control group (median: 3741 µg/kg, range: 2548-9339 µg/kg) was again highly significant ($p < 0.001$) (*Fig. 4*). Mercury was found moderately increased in 11 out of 20 cancer samples (median: 6.9 µg/kg, range: 1.8-45.9 µg/kg), a highly significant difference was recorded when compared to the control group (median: 2.1 µg/kg, range: 0.1-6.6 µg/kg) ($p < 0.005$) (*Fig. 5*). Increased cadmium concentrations have been found in 18 out of 20 cancer biopsies (median:

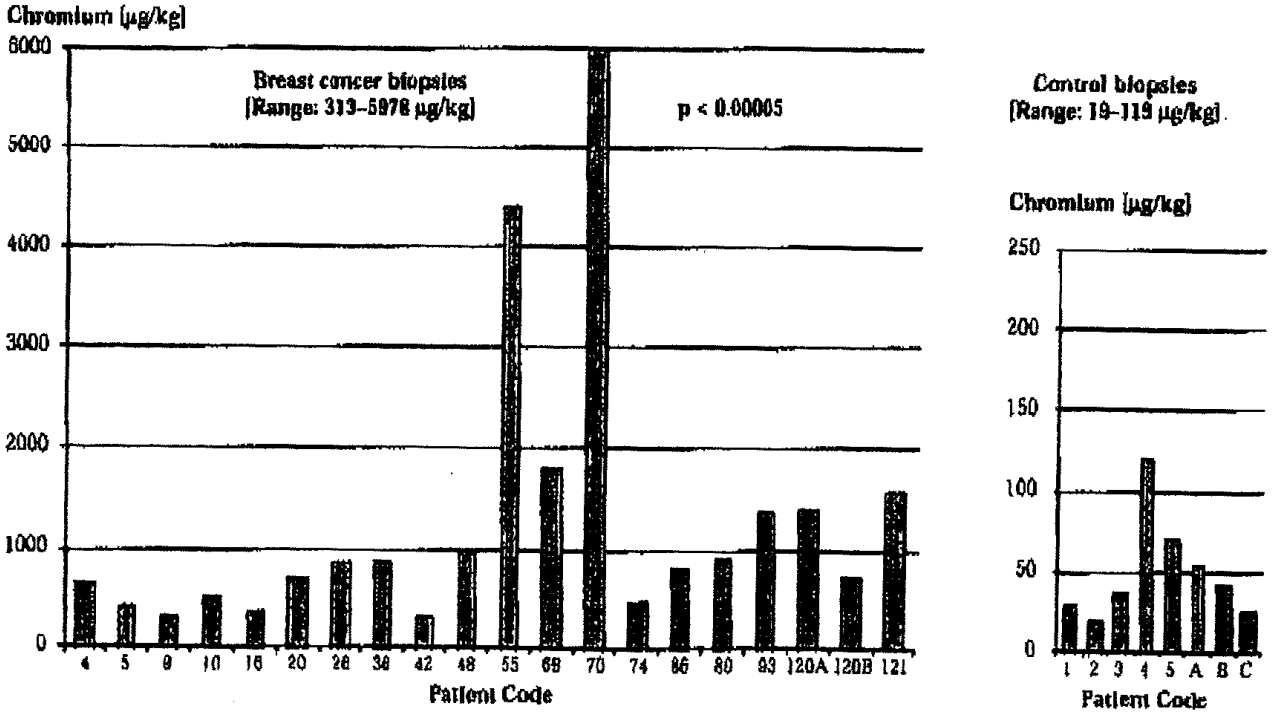

Figure 3. Chromium content of 20 breast cancer and 8 control human biopsies. Furnace-AAS technique

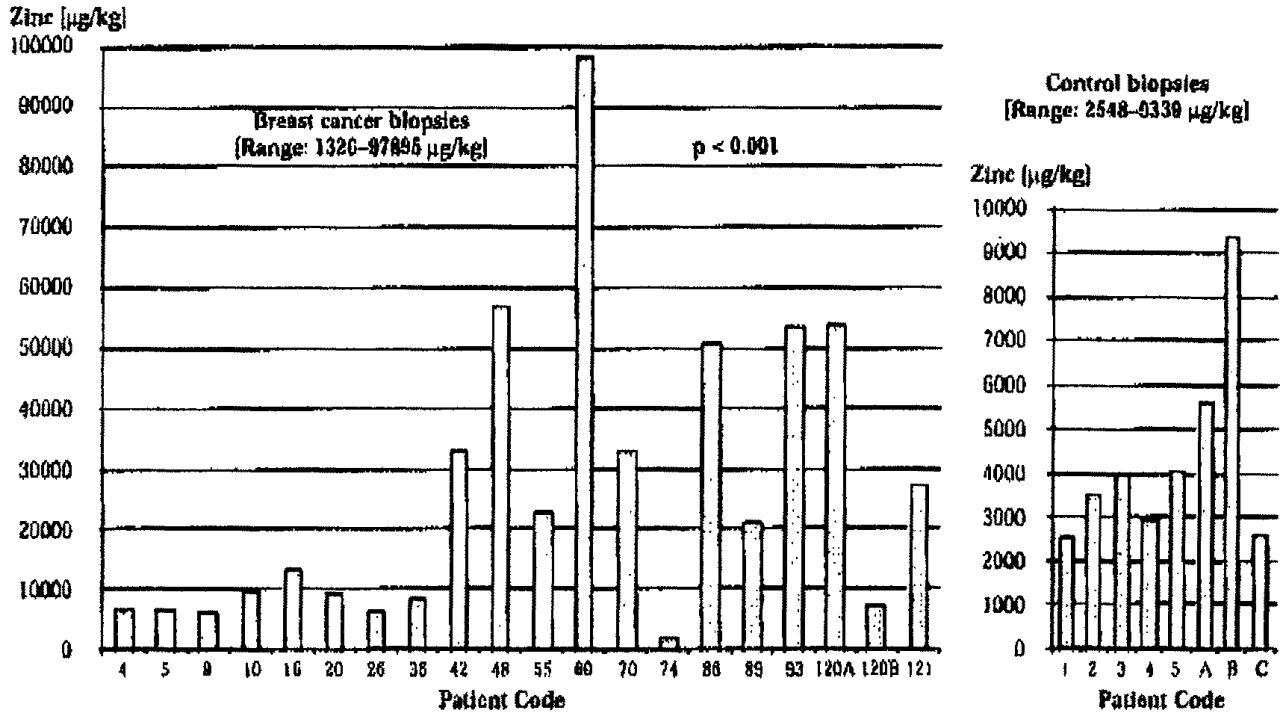

Figure 4. Zinc content of 20 breast cancer and 8 control human biopsies. Furnace-AAS technique

42 µg/kg, range: 9-551 µg/kg), the difference from the control group (median: 15.6 µg/kg, median: 5.2-30 µg/kg) was highly significant ($p < 0.005$) (*Fig. 6*). Lead was also increased in 12 out of 20 tumor biopsies (median: 104.5 µg/kg, range: 9-976 µg/kg). The difference from the control group (median: 63.5, range: 1-92 µg/kg) was still significant ($p < 0.05$).

Surprisingly, lowered copper levels were found in 11 out of 20 patient biopsies (median: 919 µg/kg, range 320-44687 µg/kg), when compared to the control samples (median: 1279.5 µg/kg, range: 261-3049 µg/kg). The other 9 cancer samples showed 7 increased values and 2 in the normal range documenting a different accumulation pattern possibly related to the tumor etiology or growth stage.

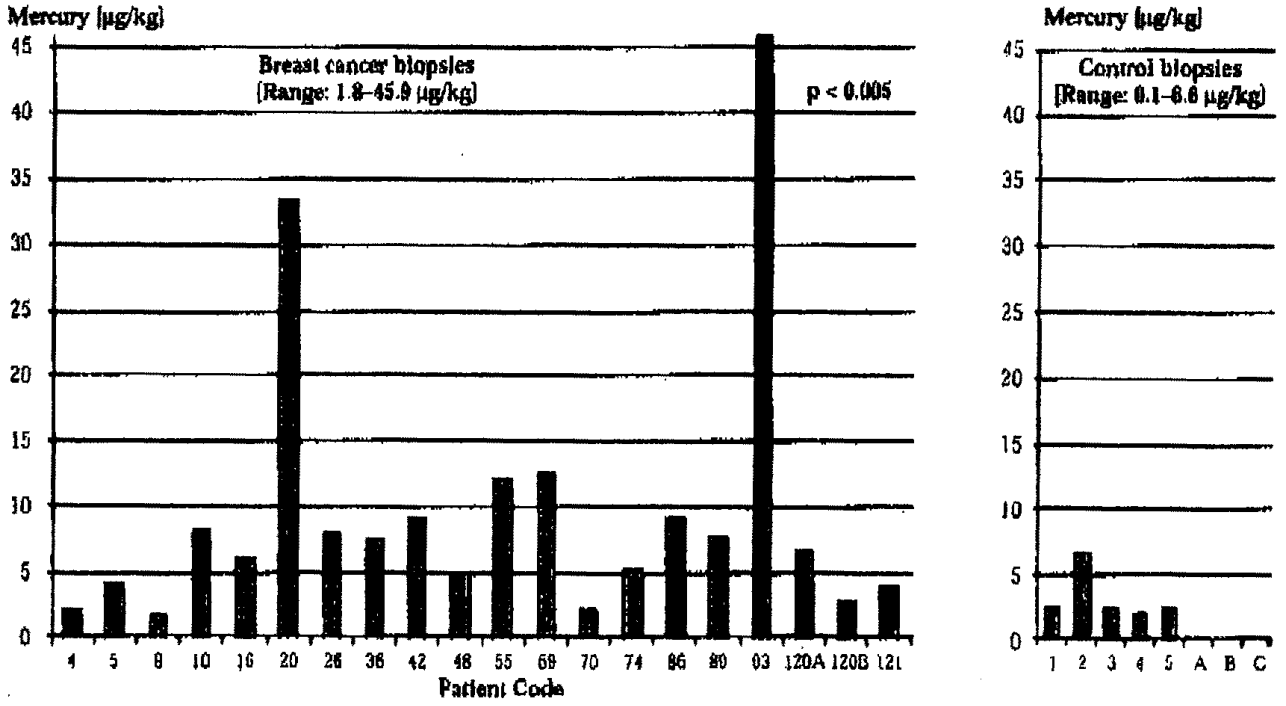

Figure 5. Mercury content of 20 breast cancer and 8 control human biopsies. Furnace-AAS technique

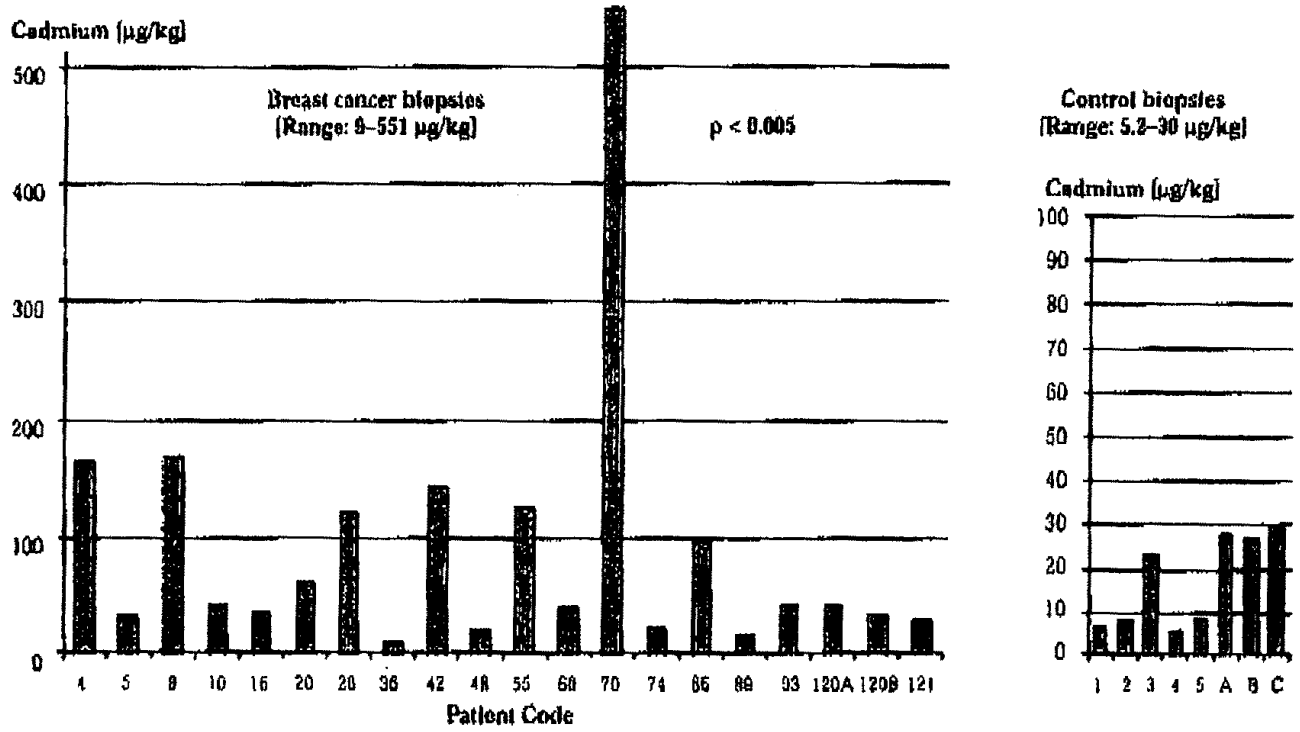

Figure 6. Cadmium content of 20 breast cancer and 8 control human biopsies. Furnace-AAS technique

Altogether, no significant difference was recorded between the cancer group and the controls ($p = 0.65$).

Only 4 out of 20 cancer samples showed detectable levels of silver (range: 34.4-90.9 µg/kg), but none of the control biopsies. Tin, gold and palladium were not detectable in cancer and control biopsies.

When compared by two different techniques (AAS and ICP-MS), there was no statistically significant difference in the heavy metal content of the control biopsies (data not shown).

Discussion

In biological systems, the concentration of redox-active transition metals capable of catalyzing/generating free radicals like superoxide, hydrogen peroxide and hydroxyl radical appears to be relatively low. However, under certain pathological conditions (hemochromatosis, Wilson disease, collagenoses and different malignancies), transition metals and their transport proteins may accumulate in different target organs, inducing cellular lipid peroxidation and DNA attack.

In this respect, the ability of excess Fe in mediating the formation of hydroxyl radicals, suppression of cellular immune functions and promotion of tumor growth is well established,[7,6,7,9] and increased Cu concentrations were also found in human lung cancer biopsies[10] and in other tumors.[11]

Ni, Cr and Cd have been recognized as mutagens and carcinogens through their ability to inhibit the repair of damaged DNA. Besides, another general feature is their property to enhance the mutagenicity and carcinogenicity of directly acting genotoxic agents.[12] At the same time, carcinogenic effects of Ni, directly or in association with organic compounds, have been described in the literature[13,14] and, recently, slightly increased concentrations of Fe and Ni have been found in malignant human prostate specimens.[15]

Inhaled particulate forms of hexavalent Cr cause lung cancer and, at cellular levels, Cr exposure may lead to cell cycle arrest, apoptosis or neoplastic transformation.[16] Occupational exposure to Cd is associated with lung cancer in humans, and high Cd concentrations were found in proliferative prostate lesions.[17] Interestingly, Zn as essential element was shown to mediate and increase tumor growth and Zn depletion was shown to suppress the tumor growth in mice and rats.[18-20] Macromolecular compounds (dextrans) substituted with Hg-containing side chains were reported to promote fibrosarcoma growth in mice.[21]

The etiology of the majority of human breast cancers is still controversial; however, hormonal influences and environmental toxic compounds inducing oxidative stress and lipid peroxidation have been suggested to play a role in breast carcinogenesis.

Our data describe for the first time a major accumulation of Fe and other transition metals like Ni, Cr, Cd, Zn, Hg and Pb in breast cancer tissue with implications in the pathogenesis of the disease.

Environmental exposure and genetic polymorphisms associated with a deficient phase II detoxification process

and alterations of metal transfer proteins or their receptors may be responsible for this phenomenon. A study of such correlations is ongoing in our facility as previous research demonstrated high levels of transferrin receptors and ferritin accumulation in breast cancer tissue.[22]

On the other hand, the higher heavy metal concentration encountered in various tumor cells may be used for therapeutic interventions with ascorbic acid or phenolic compounds as already reported.[23-25] Reduction and mobilization of transition metals from their storage or transport proteins renders them extremely reactive in catalyzing free radical reactions according to the equations:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{*}OH + OH^{-}$$

$$H_2O_2 + {}^{*}O_2^{-} \xrightarrow{Fe^{3+},\ Cu^{2+}} {}^{*}OH + OH^{-} + O_2$$

The described Fenton- and Haber-Weiss reactions are strong generators of hydroxyl radicals leading to lipid peroxidation, DNA strand breaks and apoptosis.[2,7,23] In turn, bioactivation of phenolic/quinonic compounds at the tumor site may lead to a significant generation of superoxide and semiquinone radicals with deleterious effects on the metal-rich malignant cells.[24,26] Preventive diagnostic procedures should include, besides medical imaging and current tumors markers, 2/16-OH-estrogen ratio, phase II detoxification assessment and the MELISA test ® [27] for metal specific lymphocytes.

Conclusion

The above data suggest that non-physiological gradual accumulation of transition metals in the breast tissue is strongly involved in the malignant growth process.

Acknowledgement

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